CRITICAL BODY CONCENTRATIONS: THEIR USE IN BIOINDICATION

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Abstract

A possible way to evaluate the bioavailability of pollutants in soil is to measure the concentration inside the organisms. This approach is particularly useful in the case of persistent pollutants that are retained by the organism in proportion to the concentration in the environment, and it has been applied often using heavy metals. To allow an estimation of ecological risks from residue data, information is needed on the threshold concentration in the body, above which physiological functions are irreversibly impaired. Lethal body concentrations (LBCs) may be estimated from experiments in which the increase of mortality with exposure time is observed in conjunction with the concentration of metals in the body. LBCs for cadmium have been estimated for several representatives from the soil invertebrate community These data are compared with residue analysis in two forest ecosystems in the Netherlands, to assess and compare the environmental risks of cadmium. It is proposed that the residue - LBC ratio, averaged over a number of species may be used to assess the risks of persistent toxicants at a certain site. The approach should be developed further using internal threshold concentrations for sublethal endpoints, which have a higher ecological relevance.

1. The importance of a toxicokinetic interpretation of residue data

The environmental hazard of a chemical obviously does not depend directly on its concentration in the abiotic environment (soil, water, air). Any adverse effect to the living part of the ecosystem is usually preceeded by uptake into organisms. Although there are some exceptions to this rule (*e.g.* some chemicals causing an irritating effect to the skin

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without being taken up), the concentration flux into the organism usually provides a better estimate of the chemical's hazard than the external concentration. The flux of a chemical into an animal is, however, difficult to measure, so the researcher must often be content with the measurement of a residue, which is the joint result of uptake and elimination. In many cases the residue is more or less in proportion to the rate of uptake, so it still provides a measure for uptake. In the case of chemicals with a very transient existence in the body (several pesticides), and in the case of chemicals subjected to strong physiological regulation, the residue will not be in direct proportion to the rate of uptake.

The considerations given above define the conditions under which the measurement of concentrations in the body of an animal will give an indication of its bioavailability in soil:

1. It should have a certain persistence in the body; the rate of elimination and metabolization should be in the same order of magnitude as the rate of uptake, resulting in a measurable residue.
2. It should not be regulated by the animal's physiology, in such a way that the internal concentration is independent of the external bioavailability.

Chemicals that meet these conditions are: persistent pesticides, organohalogen compounds, non-essential metals, and polycyclic aromatic hydrocarbons.

Many researchers have followed these lines of reasoning and there is a great amount of data, especially on residues of heavy metals in plants, moss bags, and invertebrates. Data on the terrestrial environment before 1980 have been collected by Martin and Coughtrey [1]. The availability of very sensitive analytical techniques (atomic absorption in a L'vov platform graphite furnace), combined with micromethods for sample preparation [2] has allowed the determination of metals in tiny soil animals, such as individual oribatid mites with a dry body mass down to 20 μg [3, 4].

The data collected for metals show a great amount of inter-species variability, which is obviously related to the physiological mechanisms underlying the storage and excretion of metals [5, 6]. A high residue may be the result of a high rate of assimilation, or a low rate of excretion, or both. Janssen [7] proposed to classify soil invertebrates in five groups, based on laboratory experiments in which uptake and elimination rates were measured for a variety of species. Data summarized in Table 1 illustrate that the measurement of fluxes is more informative than the simple determination of residues, *cf.* [8]. Some groups are relatively homogenous with respect to metal turnover (earthworms), others are very heterogeneous and great differences between species exist (oribatid mites).

TABLE 1. Overview of cadmium turnover in various taxa of soil invertebrates, classified into five groups, based on rates of uptake and elimination, according to [7]

Group	Residue	Assimilation	Excretion
1. Isopods	high	high	low
Snails	high	high	low
2. Millipedes	intermediate	intermediate	?
Centipedes	low	low	?
3. Diplurans	high	?	low
4. Collembola	low	low	high
Carabids	low	intermediate	very high
Ants	low	?	?
Crickets	low	low	high
5. Pseudoscorpions	high	high	low
Harvestmen	high	intermediate	?
Oribatid mites	intermediate/ high	intermediate/ low	low
Gamasid mites	high	high	?
Spiders	high	high	?

The collection of residue data may be useful as it provides an indication of the presence of the chemical and its bioavailability. Another reason to collect residue data is the identification of trends in time and space; many data in the aquatic environment have been collected for this purpose [9, 10]. Conclusions on the risk of a toxicant can, however, not be drawn on the basis of residues only. The species containing the highest residues are not necessarily the ones most at risk. To take the step from residue to risk, some information is needed on the internal concentrations that mark the appearance of adverse effects. This information may be derived from ecotoxicity experiments.

2. The estimation of lethal body concentrations from ecotoxicity experiments

Toxicokinetic compartment models have been very efficient in describing the changes of internal concentrations of chemicals. According to a one-compartment linear model, when test animals are given contaminated food, or are exposed to contaminated substrate, the concentration in the body will increase until an equilibrium is reached between uptake and elimination (Fig. 1). The equilibrium will be directly

proportional to the exposure concentration, and if this is high, the internal concentration is likely to approach the lethal body concentration (LBC) after some time. For low external concentrations the internal concentration will equilibrate below the LBC and the animal survives. The dynamic nature of the kinetics causes a decrease in the apparent external LC_{50}, which levels off in proportion to the rate at which the internal concentration reaches its equilibrium. Standard techniques allow explicit equations to be written for the internal body burden, Q, and the LC_{50} as a function of time (Fig. 1).

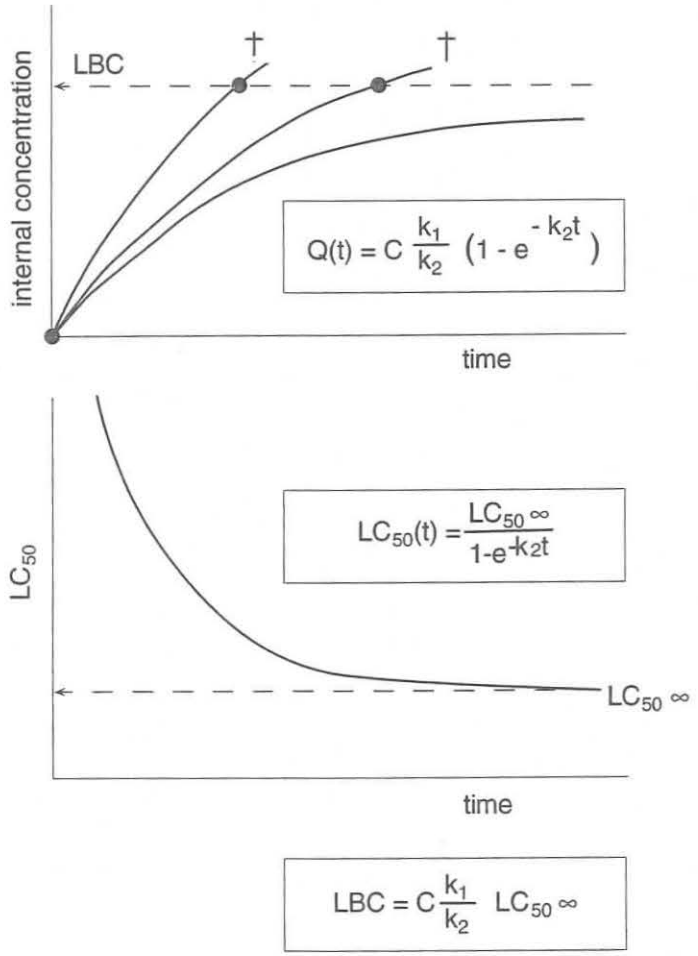

Figure 1. Graphs showing theoretical changes in the internal concentration, Q, and the external LC_{50}, as a function of time, in an ecotoxicity experiment, starting with clean test animals on time zero. The graphs follow the one-compartment toxicokinetic model, with non-zero elimination rate constant.

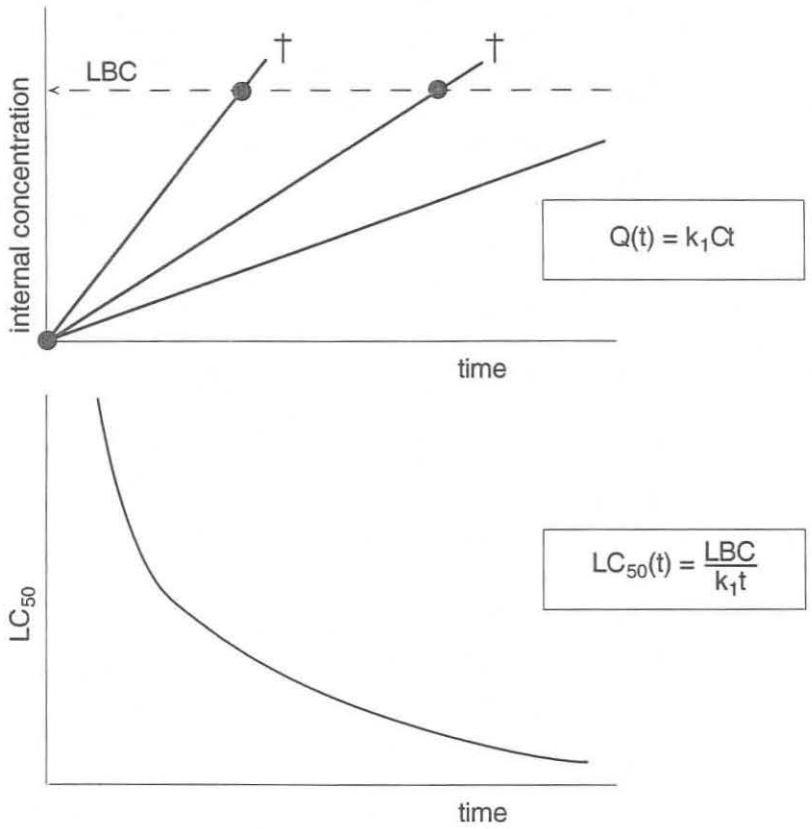

Figure 2. Graphs showing theoretical changes in the internal concentration, Q, and the external LC_{50}, as a function of time, in an ecotoxicity experiment starting with clean animals on time zero. The linear increase of Q, and the hyperbolic decrease of LC_{50} result when the elimination rate constant is zero.

A special case of the model given in Fig. 1 occurs when the elimination rate constant is zero. This is effectively the case for cadmium in isopods, pseudoscorpions, millipedes and diplurans (Table 1). According to the one-compartment model, the internal concentration then increases linearly with exposure time, and the LC_{50} does not approach a constant value, but decreases until zero. The latter prediction is due to the fact that, since every atom of metal is retained in the body, an animal will always reach a lethal body concentration, even with low exposure levels. When the exposure level is very low, the animal will, however, need a very long time to reach the LBC; if this time approaches the natural life-span of the species, the extrapolation to zero LC_{50} looses it meaning.

TABLE 2. Lethal body concentrations of cadmium for some species of soil invertebrates in ecotoxicity-toxicokinetic experiments [14]

Species	LBC (μg/g)
Orchesella cincta	37
Tomocerus minor	75
Folsomia candida	387
Platynothrus peltifer	234
Porcellio scaber	2117
Oniscus asellus	4582
Iulus scandinavius	153

The estimation of LBC from toxicokinetic data combined with ecotoxicity data, may be done using common curve-fitting techniques [11]. For the aquatic environment, LBCs have been estimated for a great variety of substances and species, especially fish [12]. For a series of homologous non-reactive lipophilic compounds, the LBC, expressed in molar units, appears to be relatively constant. This is interpretated as the body burden that causes a general syndrome of membrane damage (minimum toxicity, or non-polar narcosis, [13]). For reactive chemicals lower LBCs are found, while the lowest LBCs are seen for chemicals with a specific mode of action (*e.g.* pesticides).

For soil invertebrates, lethal body concentrations have been estimated for cadmium by Crommentuijn *et al.* [14]. Data available are reproduced in Table 2. It may be seen that LBCs are high for isopods, low for two species of springtails, and intermediate for another springtail, a mite and a millipede. The differences are very large: the isopod *Oniscus asellus* is is able to tolerate an internal body burden which is 120 times higher than the LBC of the springtail *Orchesella cincta*. In addition, there is no similarity of LBCs among taxonomically related species. The data on isopods, including the difference between *P. scaber* and *O. asellus*, are in good agreement with experiments of Hopkin [16].

4. The use of internal threshold concentrations in risk assessment

Internal threshold concentrations may be defined, not only for lethality, but also for sublethal effects. Like LBC is the internal analog of LC_{50},

ITC (internal threshold concentration) is the internal analog of the NEC (no effect concentration). Van Wensem *et al.* [15] argued that ITCs would be more invariant to environmental conditions, in comparison to NECs. The classical problem of bioavailability differences between laboratory and the field clearly holds for external concentrations, because of the use of artificial media, with unnatural speciation of the metal in laboratory experiments. For this reason, the extrapolation of NECs to field conditions is associated with great uncertainties [17]. This argument is less valid for the internal threshold concentration, because it may be assumed that an animal will be adversely affected at a fixed body burden, independent of how that body burden is reached. If this assumption is true, ecotoxicity data may be corrected for the difference in internal concentrations observed in laboratory and field, and soil standards derived from these experiments may be adjusted [15].

Ecotoxicity data for cadmium, for six species of soil invertebrates were reviewed in [15], and from the mean and standard deviation of these data, soil quality criteria were derived, using the distribution-based extrapolation methodology [17, 18]. Using the NECs from laboratory

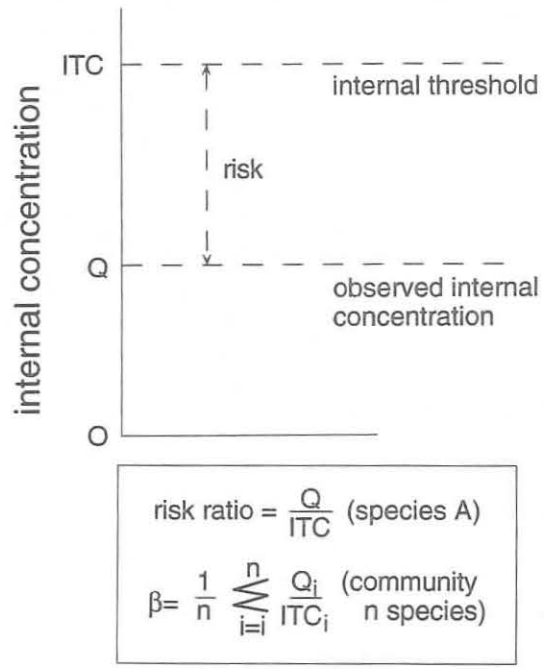

Figure 3. Schematic representation of the derivation of the "bioindicator index for toxicant residues", β, as the average (taken over a community of n species), of the quotient of the observed residue (Q) in a species, and the internal threshold concentration (ITC) for that species.

tests, a critical level of Cd in soil (hazardous concentration for 5% of the species, HC_5) was estimated as 1.7 µg/g. When corrections were applied for the difference between observed bioaccumulation factors in field studies and in laboratory experiments, the critical level became 0.24 - 0.12 µg/g, depending on whether the bioaccumulation factor was given the lowest or the highest value from an observed range. This exercise showed that biovailability differences between laboratory and field do not necessarily lead to less stringent quality criteria for soils. In this particular example, the variation between species was increased by the corrections, and this effectively decreased the estimate of HC_5.

Another approach towards the use of critical body burdens is to estimate *in situ* risks of metals. This could be done by dividing the measured residue in a certain species in the field by the internal threshold concentration for that particular species (Fig. 3). Preferably, the ratio should be determined for a number of species, and the average value of this ratio is defined here as β, the **bioindicator index for toxicant residues** (Fig. 3). It may be interpreted as the fraction of the internal threshold concentration that is occupied by the residue.

To exemplify this approach, two forest sites in the Netherlands were selected, one being contaminated due to zinc smelter emissions ("Budel"), and one acting as a reference ("Spanderswoud"). Cadmium concentrations have been measured on various occasions in these sites [3, 4, 19, 20]. For three species the data allow a comparison of LBCs and residues, see Table 3. Data in this table again illustrate that the species with the highest residue are not necessarily the ones most at risk. At the Budel site, the highest risk is actually upon the mite *P. peltifer*, which

TABLE 3. Risk assessment of two field sites ("Budel": contaminated, "Spanderswoud": reference), using the "bioindicator index for toxicant residues", β, estimated from cadmium concentrations in three species of soil invertebrates. Q = observed residue [3, 4, 19, 20]; LBC = lethal body concentration (taken from Table 2)

Species	Budel		Spanderswoud	
	Q (µg/g)	Q/LBC	Q (µg/g)	Q/LBC
Porcellio scaber	65.1	0.026	12.0	0.006
Orchesella cincta	1.50	0.041	0.36	0.010
Platynothrus peltifer	15.0	0.064	1.43	0.006
Community		β = 0.044		β = 0.007

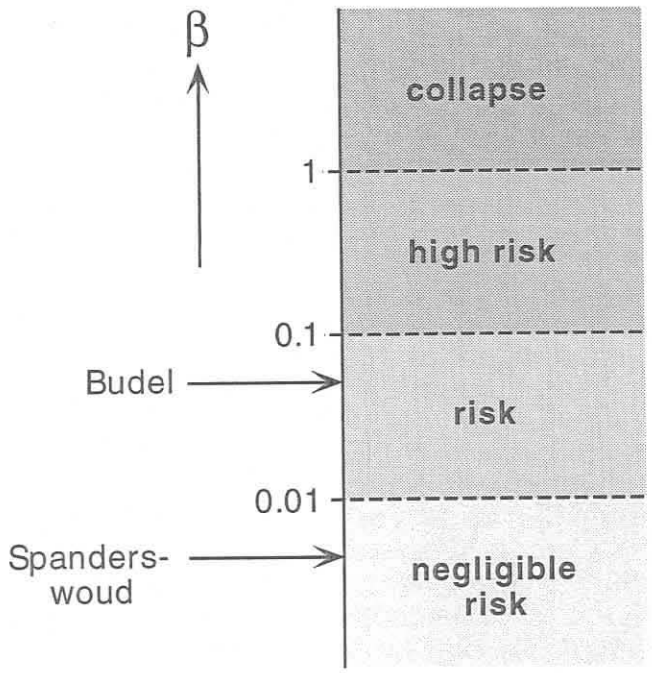

Figure 4. A proposed reference system for evaluating the bioindicator index for toxicant residues, β. The values of β at the two sites of Table 3 are indicated.

has an intermediate cadmium concentration, and not with the isopod *P. scaber*, which has the highest residue, but the lowest risk quotient.

Table 3 also shows that the interspecies variability in observed residues is in the same order of magnitude as the lethal body concentrations. The quotient of Q and LBC does not show such a great variability. On the average, 4.4 % of the LBC is occupied at the Budel site, and 0.7 % at the Spanderswoud site.

To allow an evaluation of the data presented in Table 3, one needs a system of benchmark values for β. A proposal for this is made in Fig. 4. Clearly, the highest value β can take is 1. A value of 1 would mean that, on the average, the community has its LBCs completely occupied; a total collapse of the system is expected above $\beta = 1$. The area below 0.01 is tentativily designated as "negligible risk". The benchmark of 1% follows conventions in risk assessment methodology [21]. Experimental work shows that a factor of 100 below the external LC_{50} will be

sufficient to protect from sublethal effects [22]. The area between 0.01 and 1 is arbitrarily subdivided into a "high risk" and a "risk" area, using the benchmark value of 0.1 (Fig. 4). Based on this reference system, cadmium concentrations at the site Spanderswoud would be characterized as posing a negligible risk, while the Budel site would be at risk, but not at high risk. Obviously, more comparative data are needed before such a system can be adopted as a standard reference.

4. Limitations of the approach proposed

As stated in the introduction of this chapter, the mere measurement of pollutant residues in organisms does not make sense without some reference system. I have argued here that the lethal body concentration (LBC) could serve as a reference for the observed residue to compare with. This assumes that the LBC may be considered as a constant. Some limitations must be mentioned that qualify this assumption.

1. The LBC may depend on the physiological condition of the animal, that is, an animal in a bad condition (*e.g.* due to low energy reserves) may die from an internal dose that is still tolerated by an animal in a good physiological condition [23].

2. The LBC for one metal may depend on the presence of another metal in the body. For example, cadmium may be less toxic when there is a high body burden of zinc.

3. There may be different thresholds for different parts of the body. This is not necessarily a problem, as long as all parts of the body are in dynamic equilibrium with each other. At high exposure rates, however, uptake in the dosed compartment may be quicker than the rate at which equilibrium can be reached with other compartments, and an overshoot may occur. As a consequence, the lethal dose may depend on how that dose is achieved.

4. The LBC may be subject to selection. High metal conentrations in soil may select for the most tolerant genotypes [24]. When an increase of the LBC is part of the tolerance mechanism, LBCs cannot be compared between populations of the same species living at different sites.

5. The approach assumes that the residue reflects the flux of metals through the body. This is not the case for metals that are subjected to strong regulation. For these metals the residue will be more or less constant over a broad range of exposure levels, and the LBC will be only slightly above the regulated level.

At the moment, there is insufficient information for soil invertebrates to argue which of the above mentioned considerations would seriously invalidate the LBC concept. Further research is obviously necessary, and priority should be given, not to the collection of more residue data, but

to the precise estimation of LBCs for more species of the soil invertebrate community. In addition, the approach should be developed further, using internal threshold concentrations for sublethal endpoints (growth, reproduction), which have a higher ecological relevance.

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